Serial No.: 09/826,115
Applicant: Chang, G.

Filing Date: 04/04/01
Priority Date: 06/18/01-CIP
06/03/99-PCT
06/04/98-PROV

Search Strategy

FILE 'USPATFULL' ENTERED AT 18:19:34 ON 25 NOV 2003

		E	CHANG GWONG J J/IN
L1	1	S	E4
L2	1135	S	(DENGUE VIRUS OR JAPANESE ENCEPHALITIS VIRUS OR WEST NILE VIR
L3	225	S	L2 AND (SIGNAL SEQUENCE?)
L4	22	S	L3 AND (SIGNAL SEQUENCE/CLM)
L5	29	S	L3 AND (DENGUE/CLM OR JAPANESE ENCEPHALITIS/CLM OR WEST NILE/
L6	446	S	(JAPANESE (8W) SIGNAL)
L7	1	S	L6 AND L5

FILE 'WPIDS' ENTERED AT 18:35:26 ON 25 NOV 2003

		E	CHANG G J J/IN
L8	97	S	E2
L9	2	S	L8 AND (FLAVIVIR? OR DENGUE OR JAPANESE OR YELLOW FEVER OR PO
L10	264	S	(DENGUE VIRUS OR JAPANESE ENCEPHALITIS VIRUS OR WEST NILE VIR
L11	6	S	L10 AND (SIGNAL SEQUENCE?)

FILE 'MEDLINE' ENTERED AT 18:43:44 ON 25 NOV 2003

		E	CHANG G J/AU
L12	49	S	E3
L13	16	S	L12 AND (FLAVIVIR? OR DENGUE OR YELLOW OR WEST NILE OR POWASS
L14	4970	S	(DENGUE VIRUS OR YELLOW FEVER VIRUS OR JAPANESE ENCEPHALITIS
L15	17	S	L14 AND (SIGNAL SEQUENCE)
L16	17	S	L15 NOT L12

L1 ANSWER 1 OF 1 USPATFULL on STN
2003:30900 Nucleic acid vaccines for prevention of flavivirus infection.
Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES
US 2003022849 A1 20030130
APPLICATION: US 2001-826115 A1 20010404 (9)
PRIORITY: US 1998-87908P 19980604 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention encompasses isolated nucleic acids containing transcriptional units which encode a signal sequence of one flavivirus and an immunogenic flavivirus antigen of a second flavivirus. The invention further encompasses a nucleic acid and protein vaccine and the use of the vaccine to immunize a subject against flavivirus infection. The invention also provides antigens encoded by nucleic acids of the invention, antibodies elicited in response to the antigens and use of the antigens and/or antibodies in detecting flavivirus or diagnosing flavivirus infection.

L5 ANSWER 15 OF 29 USPATFULL on STN
2002:246731 **Dengue nucleic acid vaccines that induce neutralizing antibodies.**
Kochel, Tadeusz J., Frederick, MD, United States
Porter, Kevin R., Gaithersburg, MD, United States
Raviprakash, Kanakatte, Silver Spring, MD, United States
Hoffman, Stephen L., Gaithersburg, MD, United States
Hayes, Curtis G., Frederick, MD, United States
The United States of America as represented by the Secretary of the Navy, Washington, DC, United States (U.S. government)
US 6455509 B1 20020924
APPLICATION: US 1997-869423 19970604 (8)
PRIORITY: US 1996-17839P 19960604 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A vaccine for promoting an immune response in a mammalian subject includes a eucaryotic plasmid expression vector which include at least part of the envelope gene and optionally, the PreM gene of dengue virus. In order to minimize immune enhancement, vaccines of up to the four serotypes of dengue are combined in a single vaccine. The vaccine in a suitable pharmaceutical carrier constitutes a pharmaceutical composition which is injected into the subject.

CLM What is claimed is:
1. A pharmaceutical composition capable of inducing an immune response in a mammalian subject, comprising an immunogenic amount of a eukaryotic plasmid expression vector in pharmaceutically acceptable form, wherein said plasmid expression vector is functional in mammalian subjects and includes preM and at least 92% of the envelope gene of a dengue W virus, where W is a number selected from the group consisting of 1, 2, 3 and 4.

2. The pharmaceutical composition of claim 1 further comprising a second plasmid including the PreM and at least 92% of the envelope gene of dengue X virus, where X is a number different from W and is selected from a the group consisting of 1, 2, 3 and 4.

3. The pharmaceutical composition of claim 2 further comprising a third plasmid including the PreM and at least 92% of the envelope gene of dengue Y virus, where Y is a number different from W and from X and is selected from the group consisting of 1, 2, 3 and 4.

4. The pharmaceutical composition of claim 3 further comprising a fourth plasmid including the PreM and at least 92% of the envelope gene of dengue Z virus, where Z is a number different from W, from X, and from Y, and is selected from the group consisting of 1, 2, 3 and 4.

5. The pharmaceutical composition of claim 1, further comprising a suitable pharmaceutical carrier.

6. The pharmaceutical composition of claim 5, which is in injectable form.

7. The pharmaceutical composition of claim 1, which is a vaccine capable of inducing a protective immune response in said mammalian subject, comprising an immunoprotective amount of said plasmid expression vector.

8. The vaccine of claim 7, which comprises a suitable pharmaceutical carrier, and is in injectable form.

9. A method of inducing an immune response in a mammalian subject, comprising the step of injecting the composition of claim 6.

10. A method of inducing a protective immune response in a mammalian subjects comprising the step of injecting the vaccine of claim 8.

11. The pharmaceutical composition of claim 2, further comprising a suitable pharmaceutical carrier.

12. The pharmaceutical composition of claim 11, which is in injectable form.

13. The pharmaceutical composition of claim 2, which is a vaccine capable of inducing a protective immune response in said mammalian subject, comprising an immunoprotective amount of said plasmid expression vector.

14. The vaccine of claim 13, which comprises a suitable pharmaceutical carrier, and is in injectable form.

15. A method of inducing an immune response in a mammalian subject, comprising the step of injecting the composition of claim 12.

16. A method of inducing a protective immune response in a mammalian subject comprising the step of injecting the vaccine of claim 14.

17. The pharmaceutical composition of claim 3, further comprising a suitable pharmaceutical carrier.

18. The pharmaceutical composition of claim 17, which is in injectable form.

19. The pharmaceutical composition of claim 3, which is a vaccine capable of inducing a protective immune response in said mammalian subject, comprising an immunoprotective amount of said plasmid expression vector.

20. The vaccine of claim 19, which comprises a suitable pharmaceutical carrier, and is in injectable form.

21. A method of inducing an immune response in a mammalian subject, comprising the step of injecting the composition of claim 18.

22. A method of inducing a protective immune response in a mammalian subject, comprising the step of injecting the vaccine of claim 20.

23. The pharmaceutical composition of claim 4, further comprising a suitable pharmaceutical carrier.

24. The pharmaceutical composition of claim 23, which is in injectable form.

25. The pharmaceutical composition of claim 4, which is a vaccine capable of inducing a protective immune response in said mammalian subject, comprising an immunoprotective amount of said plasmid expression vector.

26. The vaccine of claim 25, which comprises a suitable pharmaceutical carrier, and is in injectable form.

27. A method of inducing an immune response in a mammalian subject, comprising the step of injecting the composition of claim 24.

28. A method of inducing a protective immune response in a mammalian subject, comprising the step of injecting the vaccine of claim 26.

L5 ANSWER 19 OF 29 USPATFULL on STN
2000:174106 **Subunit immonogenic composition against dengue infection.**
Ivy, John, Kailua, HI, United States
Nakano, Eilen, Hon., HI, United States
Clements, David, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6165477 20001226
APPLICATION: US 1997-915152 19970820 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The Flaviviridae comprise a number of medically important pathogens that cause significant morbidity in humans including the dengue (DEN) virus, Japanese encephalitis (JE) virus, tick-borne encephalitis virus (TBE), and yellow fever virus (YF). Flaviviruses are generally transmitted to vertebrates by chronically infected mosquito or tick vectors. The viral particle which is enveloped by host cell membranes, comprises a single positive strand genomic RNA and the structural capsid (CA), membrane (M), and envelope (E) proteins. The E and M proteins are found on the surface of the virion where they are anchored in the membrane. Mature E is glycosylated and contains functional domains responsible for cell surface attachment and intraendosomal fusion activities. Problems have arisen in the art with respect to producing recombinant forms of the E glycoprotein that retain their native configuration and attendant properties associated therewith (i.e., ability to induce neutralizing antibody responses). To date, recombinantly produced E glycoproteins have suffered from a number of limitations including improper glycosylation, folding, and disulfide bond formation. The claimed invention has addressed these concerns by providing secreted recombinant forms of the E glycoprotein that are highly immunogenic and appear to retain their native configuration. Carboxy-terminally truncated forms of E containing the amino terminal 395 amino acids and a suitable secretion signal sequence were generated in Drosophila melanogaster Schneider cell lines. Immunogenic compositions comprising these recombinant

envelope glycoproteins were capable of inducing protective, neutralizing antibody responses when administered to a suitable host.

CLM What is claimed is:

1. An immunogenic composition which generates protective, neutralizing antibody responses to a Flavivirus in a murine host which responses confer protection against intracerebral challenge by the homologous Flavivirus, said strain of Flavivirus selected from the group consisting of a strain of dengue, a strain of Japanese encephalitis virus (JEV), a strain of yellow fever virus (YF), and a strain of tick-borne encephalitis virus (TBE) which composition contains an adjuvant; and a portion of the envelope protein (E) of the Flavivirus strain against which said responses are sought, which portion is 80% E, wherein said 80% E represents that portion of the envelope protein that constitutes 80% of its length starting from amino acid 1 at its N-terminus and which portion has been secreted as a recombinantly produced protein from Drosophila cells.

2. The immunogenic composition of claim 1 wherein said Drosophila cells are D. melanogaster Schneider cells.

3. The immunogenic composition of claim 1 wherein said adjuvant is an alum adjuvant.

4. An immunogenic composition which generates a neutralizing antibody response to a Flavivirus in a murine host against the homologous Flavivirus, said strain of Flavivirus selected from the group consisting of a strain of dengue, a strain of Japanese encephalitis virus (JEV), a strain of yellow fever virus (YF), and a strain of tick-borne encephalitis virus (TBE) which composition contains an adjuvant; and a portion of the envelope protein (E) of the Flavivirus strain against which generation of said response is sought, which portion is 80% E, wherein said 80% E represents that portion of the envelope protein that constitutes 80% of its length starting from amino acid 1 at its N-terminus, and which portion has been secreted as a recombinantly produced protein from Drosophila cells.

5. The immunogenic composition of claim 4 wherein said Drosophila cells are Schneider cells.

6. The immunogenic composition of claim 4 wherein said Flavivirus is a dengue virus.

7. The immunogenic composition of claim 4 wherein the 80% E is encoded in a DNA construct operably linked downstream from human tissue plasminogen activator prepropeptide secretion leader (tPA.sub.L).

8. The immunogenic composition of claim 4 wherein the adjuvant is an alum adjuvant.

9. A method to generate a neutralizing antibody response in a non-human subject against a Flavivirus strain, said strain selected from the group consisting of a strain of dengue, a strain of YF, a strain of JEV, and a strain of TBE, which method comprises administering to a non-human subject in need of generating said response an effective amount of the immunogenic composition of claim 4.

10. The method of claim 9 wherein said Flavivirus is a dengue virus.

11. The immunogenic composition of claim 1 wherein the 80% E is encoded in a DNA construct operably linked downstream from a **human tissue plasminogen activator prepropeptide secretion leader (tPA.sub.L) sequence.**

12. The immunogenic composition of claim 1 wherein said Flavivirus is a dengue virus.

13. The immunogenic composition of claim 2 wherein the Flavirirus is a dengue virus.

L5 ANSWER 20 OF 29 USPATFULL on STN
2000:142128 **Methods of preparing carboxy-terminally truncated recombinant flavivirus envelope glycoproteins employing drosophila melanogaster expression systems.**
Ivy, John, Kailua, HI, United States
Nakano, Eilen, Honolulu, HI, United States
Clements, David, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6136561 20001024
APPLICATION: US 1997-937195 19970925 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The Flaviviridae comprise a number of medically important pathogens that cause significant morbidity in humans including the dengue (DEN) virus, Japanese encephalitis (JE) virus, tick-borne encephalitis virus (TBE), and yellow fever virus (YF). Flaviviruses are generally transmitted to vertebrates by chronically infected mosquito or tick vectors. The viral particle which is enveloped by host cell membranes, comprises a single positive strand genomic RNA and the structural capsid (CA), membrane (M), and envelope (E) proteins. The E and M proteins are found on the surface of the virion where they are anchored in the membrane. Mature E is glycosylated and contains functional domains responsible for cell surface attachment and intraendosomal fusion activities. Problems have arisen in the art with respect to producing recombinant forms of the E glycoprotein that retain their native configuration and attendant properties associated therewith (i.e., ability to induce neutralizing antibody responses). To date, recombinantly produced E glycoproteins have suffered from a number of limitations including improper glycosylation, folding, and disulfide bond formation. The claimed invention has addressed these concerns by providing secreted recombinant forms of the E glycoprotein that are highly immunogenic and appear to retain their native configuration. Carboxy-terminally truncated forms of E containing the amino terminal 395 amino acids and a suitable secretion signal sequence were generated in Drosophila melanogaster Schneider cell lines. The recombinant proteins produced by this expression system should prove useful, inter alia, as immunogens and diagnostic reagents.

CLM What is claimed is:
1. An expression system for the recombinant production and secretion of a portion of an envelope (E) protein of a Flavivirus selected from the group consisting of dengue virus, Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBE) and yellow fever virus (YF), which expression system comprises Drosophila cells modified to contain a

DNA molecule which comprises (a) a first nucleotide sequence encoding said portion of said E protein of the Flavivirus strain against which protection is sought, which portion is the N-terminal 80% of the protein from residue 1 to residue 395, and (b) a second nucleotide sequence which encodes a secretory leader sequence or a secretory **signal sequence** operably linked to said first nucleotide sequence and positioned so as to produce a fusion protein when said first and said second nucleotide sequences are expressed in a eucaryotic cell, said encoding sequences operably linked to control sequences capable of effecting expression of said encoding nucleotide sequences in eucaryotic cells.

2. The expression system of claim 1 wherein said secretory leader sequence is human tissue plasminogen activator prepropeptide secretion leader (tPA.sub.L) and optionally includes the premembrane leader of the E protein.

3. A method to produce a portion of an E protein of a Flavivirus selected from the group consisting of dengue virus, Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBE) and yellow fever virus (YF), which method comprises (a) culturing the Drosophila cells of claim 1 in culture medium under conditions favorable for expression of the encoding nucleotide sequence so that the cells secrete said portion of the E protein of the Flavivirus strain against which protection is sought, which portion is the N-terminal 80% of the protein from residue 1 to residue 395 into the medium; and (b) recovering the portion of the E protein from the culture medium.

4. A method to produce a portion of an E protein of a Flavivirus selected from the group consisting of dengue virus, Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBE) and yellow fever virus (YF), which method comprises (a) culturing the Drosophila cells of claim 2 in culture medium under conditions favorable for expression of the encoding nucleotide sequence so that the cells secrete said portion of the E protein of the Flavivirus strain against which protection is sought, which portion is the N-terminal 80% of the protein from residue 1 to residue 395 into the medium; and (b) recovering the portion of the E protein from the culture medium.

5. The expression system of claim 1 wherein the N-terminal 80% of the E protein from residue 1 to residue 395 is dengue virus E protein.

6. The method of claim 3 wherein the N-terminal 80% of the E protein from residue 1 to residue 395 is dengue virus E protein.

7. The method of claim 4 wherein the N-terminal 80% of the E protein from residue 1 to residue 395 is dengue virus E protein.

8. The expression system of claim 1, wherein the Drosophila cells are Drosophila Schneider cells.

9. The expression system of claim 2, wherein the Drosophila cells are Drosophila Schneider cells.

10. The method of claim 3, wherein the Drosophila cells are Drosophila Schneider cells.

11. The method of claim 4, wherein the Drosophila cells are Drosophila Schneider cells.

12. The expression system of claim 5, wherein the Drosophila cells are Drosophila Schneider cells.

13. The method of claim 6, wherein the Drosophila cells are Drosophila Schneider cells.

14. The method of claim 7, wherein the Drosophila cells are Drosophila Schneider cells.

L9 ANSWER 2 OF 2 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
AN 2000-072885 [06] WPIDS
CR 2003-058572 [05]
DNC C2000-020998
TI Novel nucleic acid for use in vaccines.
DC B04 D16
IN CHANG, G J
PA (USSH) US DEPT HEALTH & HUMAN SERVICES; (USSH) US CENTERS DISEASE CONTROL & PREVENTION; (CHAN-I) CHANG G J
CYC 86
PI WO 9963095 A1 19991209 (200006)* EN 58p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
AU 9943296 A 19991220 (200021)
BR 9910830 A 20010213 (200114)
EP 1084252 A1 20010321 (200117) EN
R: DE FR GB NL
JP 2002517200 W 20020618 (200242) 63p
US 2003022849 A1 20030130 (200311)
ADT WO 9963095 A1 WO 1999-US12298 19990603; AU 9943296 A AU 1999-43296 19990603; BR 9910830 A BR 1999-10830 19990603, WO 1999-US12298 19990603; EP 1084252 A1 EP 1999-955295 19990603, WO 1999-US12298 19990603; JP 2002517200 W WO 1999-US12298 19990603, JP 2000-552289 19990603; US 2003022849 A1 Provisional US 1998-87908P 19980604, CIP of WO 1999-US12298 19990603, US 2001-826115 20010404, CIP of US 2001-701536 20010618
FDT AU 9943296 A Based on WO 9963095; BR 9910830 A Based on WO 9963095; EP 1084252 A1 Based on WO 9963095; JP 2002517200 W Based on WO 9963095
PRAI US 1998-87908P 19980604; US 2001-826115 20010404; US 2001-701536 20010618

AB **WO 9963095** A UPAB: 20030214
NOVELTY - Nucleic acid molecule (I) comprises a transcription unit (TU) for an immunogenic flavivirus antigen (Ag). When incorporated into a host cell, TU directs synthesis of Ag.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) host cells containing (I); and
(2) vaccines containing (I) plus a carrier.
ACTIVITY - Antiviral.
MECHANISM OF ACTION - Vaccine.
USE - (I) are used in vaccines to protect against flavivirus infection. Also (not claimed) (I) can be used to produce Ag for analytical or diagnostic applications. Plasmid pCBJE1-14 contains a fragment of nucleic acid encoding the pre-M and E proteins of Japanese

encephalitis virus (JEV) cloned into pCBamp. It was administered intramuscularly (50-100 mu g) to 3-day old mice. After 7 weeks all animals were seropositive for JEV and all were protected against subsequent challenge by the mouse-adapted SA14 strain of JEV (contrast 40% survival for animals inoculated with the commercial vaccine JE-VAX).

ADVANTAGE - (I) makes possible inexpensive and safe production of a storage-stable vaccine that has minimal risk of causing adverse immunological reactions to impurities. The vaccines elicit neutralizing antibodies and protective immunity very effectively (i.e. 100% protection), and since they contain only part of the viral genome they can not cause infection in those manufacturing or receiving them. The immunity conferred by the vaccine is transmitted to offspring through the milk.

Dwg.0/8

L13 ANSWER 1 OF 16 MEDLINE on STN

2002071990 Document Number: 21656052. PubMed ID: 11797784. Flavivirus DNA vaccines: current status and potential. Chang G J; Davis B S; Hunt A R; Holmes D A; Kuno G. (Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado 80522, USA.. gxc7@cdc.gov) . ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (2001 Dec) 951 272-85. Ref: 54. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.

AB The use of DNA-based vaccines is a novel and promising immunization approach for the development of flavivirus vaccines. This approach has been attempted in vaccine development for various virus species, including St. Louis encephalitis, Russian spring-summer encephalitis, Central European encephalitis, dengue serotypes 1 and 2, Murray Valley encephalitis, Japanese encephalitis, and West Nile viruses. However, very little is known about the factors affecting its efficacy. Recently, we demonstrated that a single intramuscular immunization of DNA vaccine of Japanese encephalitis and West Nile viruses protected mice and horses from virus challenge. Administration of these recombinant plasmid vectors resulted in endogenous expression and secretion of extracellular virus-like particles that correlated well with the induction of protective immunity. These results provided evidence that the virus-like particles composed of premembrane/membrane and envelope proteins are essential for eliciting immune responses similar to those induced by live, attenuated virus vaccines. The biosynthesis and protein processing of premembrane/membrane and envelope proteins that preserve the native conformation and glycosylation profiles identical to virion proteins could be determined by the effectiveness of the transmembrane signal sequence located at the amino-terminus of premembrane protein. The use of DNA vaccines in multivalent and/or combination vaccines designed to immunize against multiple flaviviruses is also a promising area of development.

L13 ANSWER 2 OF 16 MEDLINE on STN

2001670768 Document Number: 21572221. PubMed ID: 11716135. Genomic sequencing of deer tick virus and phylogeny of powassan-related viruses of North America. Kuno G; Artsob H; Karabatsos N; Tsuchiya K R; Chang G J. (Division of Vector-Borne Infectious Diseases, National Institute of Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado 80522, USA.. gok1@cdc.gov) . AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, (2001 Nov) 65 (5) 671-6. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB **Powassan (POW) virus is responsible for central nervous system**

infection in humans in North America and the eastern parts of Russia. Recently, a new flavivirus, deer tick (DT) virus, related to POW virus was isolated in the United States, but neither its pathogenic potential in human nor the taxonomic relationship with POW virus has been elucidated. In this study, we obtained the near-full-length genomic sequence of the DT virus and complete sequences of 3 genomic regions of 15 strains of POW-related virus strains. The phylogeny revealed 2 lineages, one of which had the prototype POW virus and the other DT virus. Both lineages can cause central nervous system infection in humans. By use of the combination of molecular definition of virus species within the genus Flavivirus and serological distinction in a 2-way cross-neutralization test, the lineage of DT virus is classified as a distinct genotype of POW virus.

L13 ANSWER 3 OF 16 MEDLINE on STN
2001413886 Document Number: 21356882. PubMed ID: 11463410. Fever and multisystem organ failure associated with 17D-204 yellow fever vaccination: a report of four cases. Martin M; Tsai T F; Cropp B; Chang G J; Holmes D A; Tseng J; Shieh W; Zaki S R; Al-Sanouri I; Cutrona A F; Ray G; Weld L H; Cetron M S. (Divisions of Global Migration and Quarantine, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.) LANCET, (2001 Jul 14) 358 (9276) 98-104. Journal code: 2985213R. ISSN: 0140-6736. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: In 1998, the US Centers for Disease Control and Prevention was notified of three patients who developed severe illnesses days after yellow fever vaccination. A similar case occurred in 1996. All four patients were more than 63 years old. METHODS: Vaccine strains of yellow fever virus, isolated from the plasma of two patients and the cerebrospinal fluid of one, were characterised by genomic sequencing. Clinical samples were subjected to neutralisation assays, and an immunohistochemical analysis was done on one sample of liver obtained at biopsy. FINDINGS: The clinical presentations were characterised by fever, myalgia, headache, and confusion, followed by severe multisystemic illnesses. Three patients died. Vaccine-related variants of yellow fever virus were found in plasma and cerebrospinal fluid of one vaccinee. The convalescent serum samples of two vaccinees showed antibody responses of at least 1:10240. Immunohistochemical assay of liver tissue showed yellow fever antigen in the Kuppfer cells of the liver sample. INTERPRETATION: The clinical features, their temporal association with vaccination, recovery of vaccine-related virus, antibody responses, and immunohistochemical assay collectively suggest a possible causal relation between the illnesses and yellow fever vaccination. Yellow fever remains an important cause of illness and death in South America and Africa; hence, vaccination should be maintained until the frequency of these events is quantified.

L13 ANSWER 4 OF 16 MEDLINE on STN
2001200576 Document Number: 21184685. PubMed ID: 11287553. West Nile virus recombinant DNA vaccine protects mouse and horse from virus challenge and expresses in vitro a noninfectious recombinant antigen that can be used in enzyme-linked immunosorbent assays. Davis B S; Chang G J; Cropp B; Roehrig J T; Martin D A; Mitchell C J; Bowen R; Bunning M L. (Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services, Fort Collins, Colorado 80522, USA.) JOURNAL OF VIROLOGY, (2001 May) 75 (9) 4040-7. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Introduction of West Nile (WN) virus into the United

States in 1999 created major human and animal health concerns. Currently, no human or veterinary vaccine is available to prevent WN viral infection, and mosquito control is the only practical strategy to combat the spread of disease. Starting with a previously designed eukaryotic expression vector, we constructed a recombinant plasmid (pCBWN) that expressed the WN virus prM and E proteins. A single intramuscular injection of pCBWN DNA induced protective immunity, preventing WN virus infection in mice and horses. Recombinant plasmid-transformed COS-1 cells expressed and secreted high levels of WN virus prM and E proteins into the culture medium. The medium was treated with polyethylene glycol to concentrate proteins. The resultant, containing high-titered recombinant WN virus antigen, proved to be an excellent alternative to the more traditional suckling-mouse brain WN virus antigen used in the immunoglobulin M (IgM) antibody-capture and indirect IgG enzyme-linked immunosorbent assays. This recombinant antigen has great potential to become the antigen of choice and will facilitate the standardization of reagents and implementation of WN virus surveillance in the United States and elsewhere.

L13 ANSWER 5 OF 16 MEDLINE on STN
2000173690 Document Number: 20173690. PubMed ID: 10708416. Chimeric dengue type 2 (vaccine strain PDK-53)/dengue type 1 virus as a potential candidate dengue type 1 virus vaccine. Huang C Y; Butrapet S; Pierro D J; Chang G J; Hunt A R; Bhamarapravati N; Gubler D J; Kinney R M. (Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Fort Collins, Colorado 80522, USA.) JOURNAL OF VIROLOGY, (2000 Apr) 74 (7) 3020-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We constructed chimeric dengue type 2/type 1 (DEN-2/DEN-1) viruses containing the nonstructural genes of DEN-2 16681 virus or its vaccine derivative, strain PDK-53, and the structural genes (encoding capsid protein, premembrane protein, and envelope glycoprotein) of DEN-1 16007 virus or its vaccine derivative, strain PDK-13. We previously reported that attenuation markers of DEN-2 PDK-53 virus were encoded by genetic loci located outside the structural gene region of the PDK-53 virus genome. Chimeric viruses containing the nonstructural genes of DEN-2 PDK-53 virus and the structural genes of the parental DEN-1 16007 virus retained the attenuation markers of small plaque size and temperature sensitivity in LLC-MK(2) cells, less efficient replication in C6/36 cells, and attenuation for mice. These chimeric viruses elicited higher mouse neutralizing antibody titers against DEN-1 virus than did the candidate DEN-1 PDK-13 vaccine virus or chimeric DEN-2/DEN-1 viruses containing the structural genes of the PDK-13 virus. Mutations in the envelope protein of DEN-1 PDK-13 virus affected in vitro phenotype and immunogenicity in mice. The current PDK-13 vaccine is the least efficient of the four Mahidol candidate DEN virus vaccines in human trials. The chimeric DEN-2/DEN-1 virus might be a potential DEN-1 virus vaccine candidate. This study indicated that the infectious clones derived from the candidate DEN-2 PDK-53 vaccine are promising attenuated vectors for development of chimeric flavivirus vaccines.

L13 ANSWER 7 OF 16 MEDLINE on STN
1998080391 Document Number: 98080391. PubMed ID: 9420202. Phylogeny of the genus Flavivirus. Kuno G; Chang G J; Tsuchiya K R; Karabatsos N; Cropp C B. (Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado 80522-2087, USA.. GOK1@CDC.GOV) . JOURNAL OF VIROLOGY, (1998 Jan) 72 (1) 73-83. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We undertook a comprehensive phylogenetic study to establish the genetic relationship among the viruses of the genus Flavivirus and to compare the classification based on molecular phylogeny with the existing serologic method. By using a combination of quantitative definitions (bootstrap support level and the pairwise nucleotide sequence identity), the viruses could be classified into clusters, clades, and species. Our phylogenetic study revealed for the first time that from the putative ancestor two branches, non-vector and vector-borne virus clusters, evolved and from the latter cluster emerged tick-borne and mosquito-borne virus clusters. Provided that the theory of arthropod association being an acquired trait was correct, pairwise nucleotide sequence identity among these three clusters provided supporting data for a possibility that the non-vector cluster evolved first, followed by the separation of tick-borne and mosquito-borne virus clusters in that order. Clades established in our study correlated significantly with existing antigenic complexes. We also resolved many of the past taxonomic problems by establishing phylogenetic relationships of the antigenically unclassified viruses with the well-established viruses and by identifying synonymous viruses.

L16 ANSWER 1 OF 17 MEDLINE on STN
2003245150 Document Number: 22652828. PubMed ID: 12768036. Flavivirus capsid is a dimeric alpha-helical protein. Jones Christopher T; Ma Lixin; Burgner John W; Groesch Teresa D; Post Carol B; Kuhn Richard J. (Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, USA.) JOURNAL OF VIROLOGY, (2003 Jun) 77 (12) 7143-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The capsid proteins of two flaviviruses, yellow fever virus and dengue virus, were expressed in Escherichia coli and purified to near homogeneity suitable for biochemical characterization and structure determination by nuclear magnetic resonance. The oligomeric properties of the capsid protein in solution were investigated. In the absence of nucleic acid, both proteins were predominantly dimeric in solution. Further analysis of both proteins with far-UV circular dichroism spectroscopy indicated that they were largely alpha-helical. The secondary structure elements of the dengue virus capsid were determined by chemical shift indexing of the sequence-specific backbone resonance assignments. The dengue virus capsid protein devoid of its C-terminal signal sequence was found to be composed of four alpha helices. The longest alpha helix, 20 residues, is located at the C terminus and has an amphipathic character. In contrast, the N terminus was found to be unstructured and could be removed without disrupting the structural integrity of the protein.

L16 ANSWER 2 OF 17 MEDLINE on STN
2002200336 Document Number: 21848592. PubMed ID: 11858863. Protective efficacy of a plasmid DNA encoding Japanese encephalitis virus envelope protein fused to tissue plasminogen activator signal sequences: studies in a murine intracerebral virus challenge model. Ashok Mundrigri S; Rangarajan Pundi N. (Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India.) VACCINE, (2002 Feb 22) 20 (11-12) 1563-70. Journal code: 8406899. ISSN: 0264-410X. Pub. country: England: United Kingdom. Language: English.

AB We report the construction of chimeric DNA vaccine vectors in which secretory signal sequence derived from tissue plasminogen activator (TPA) was fused to the full length (pCMVTE) or 398 amino terminal amino acids (pCMVTdeltaE) of Japanese

encephalitis virus (JEV) envelope (E) protein.
Transfection studies indicate that E protein expressed from pCMVTdeltaE-transfected cells but not pCMVTE-transfected cells is secreted into the culture medium. Analysis of the potency of various DNA vaccine constructs in a murine intracerebral (i.c.) JEV challenge model indicates that pCMVTdeltaE confers the highest level (71%) of protection. Immunization with pCMVTdeltaE induces a mixed Th1 and Th2 T helper cell response while immunization with plasmids encoding nonsecretory forms of E protein induces a Th1 T helper response. Only low levels (<1:20) of virus neutralizing antibody titres were observed in DNA vaccinated mice which did not increase further after i.c. JEV challenge. Thus, immunization with a plasmid encoding secretory E protein results in an altered cytokine response and better protection against i.c. JEV challenge than that conferred by immunization with plasmids encoding nonsecretory forms of E protein. We also demonstrate that unlike peripheral JEV challenge, i.c. JEV challenge does not result in an increase in anamnestic antibody response suggesting that other components of immune system such as cytotoxic T cells and T helper cells contribute to protection against i.c. JEV challenge of DNA vaccinated mice.

L16 ANSWER 3 OF 17 MEDLINE on STN
2000059995 Document Number: 20059995. PubMed ID: 10590335. A DNA vaccine expressing dengue type 2 virus premembrane and envelope genes induces neutralizing antibody and memory B cells in mice. Konishi E; Yamaoka M; Kurane I; Mason P W. (Department of Health Sciences, Kobe University School of Medicine, 7-10-2 Tomogaoka, Suma-ku, Kobe, Japan.. ekon@ams.kobe-u.ac.jp) . VACCINE, (2000 Jan 6) 18 (11-12) 1133-9. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A dengue DNA vaccine candidate was developed and evaluated for immunogenicity in mice. The vaccine, designated pcD2ME, is a pcDNA3-based plasmid encoding the **signal sequence of premembrane (prM)**, prM and envelope (E) genes of the New Guinea C strain of dengue type 2 virus. CHO-K1 cells transfected with pcD2ME expressed prM and E as determined by immunochemical staining with monoclonal antibodies. BALB/c mice inoculated intramuscularly with 100 microg of pcD2ME two or three times at an interval of 2 weeks developed a low level of neutralizing antibody (1:10 at a 90% plaque reduction). Immunization twice with 10 microg or 1 microg of pcD2ME or three times with 100 microg of pcDNA3 did not induce detectable levels of neutralizing antibody. Mice immunized two or three times with 100 microg of pcD2ME raised neutralizing antibody titers to 1:40 or greater on days 4 and 8 after challenge with 3x10(5) plaque forming units (PFU) of the New Guinea C strain of dengue type 2 virus, showing strong anamnestic responses to the challenge. In contrast, mice immunized two or three times with 100 microg of pcDNA3 developed no detectable neutralizing antibody on days 4 and 8 after challenge. These results indicate that immunization with pcD2ME induces neutralizing antibody and dengue type 2 virus-responsive memory B cells in mice.

L16 ANSWER 4 OF 17 MEDLINE on STN
2000057892 Document Number: 20057892. PubMed ID: 10590087. **Mutagenesis of the signal sequence of yellow fever virus prM protein: enhancement of signalase cleavage In vitro is lethal for virus production. Lee E; Stocks C E; Amberg S M; Rice C M; Lobigs M. (Division of Immunology and Cell Biology, John Curtin School of Medical Research, The Australian National University, Canberra, Australian Capital Territory 2601, Australia.) JOURNAL OF VIROLOGY, (2000 Jan) 74 (1) 24-32. Journal code: 0113724. ISSN: 0022-538X.** Pub. country: United States. Language: English.

AB Proteolytic processing at the C-prM junction in the flavivirus polyprotein involves coordinated cleavages at the cytoplasmic and luminal sides of an internal signal sequence. We have introduced at the COOH terminus of the yellow fever virus (YFV) **prM signal sequence** amino acid substitutions (VPQAQA mutation) which uncoupled efficient signal peptidase cleavage of the prM protein from its dependence on prior cleavage in the cytoplasm of the C protein mediated by the viral NS2B-3 protease. Infectivity assays with full-length YFV RNA transcripts showed that the VPQAQA mutation, which enhanced signal peptidase cleavage in vitro, was lethal for infectious virus production. Revertants or second-site mutants were recovered from cells transfected with VPQAQA RNA. Analysis of these viruses revealed that single amino acid substitutions in different domains of the prM signal sequence could restore viability. These variants had growth properties in vertebrate cells which differed only slightly from those of the parent virus, despite efficient signal peptidase cleavage of prM in cell-free expression assays. However, the neurovirulence in mice of the VPQAQA variants was significantly attenuated. This study demonstrates that substitutions in the prM signal sequence which disrupt coordinated cleavages at the C-prM junction can impinge on the biological properties of the mutant viruses. Factors other than the rate of production of prM are vitally controlled by regulated cleavages at this site.

L16 ANSWER 5 OF 17 MEDLINE on STN
1998033180 Document Number: 98033180. PubMed ID: 9367357. **Analysis of a recombinant dengue-2 virus-dengue-3 virus hybrid envelope protein expressed in a secretory baculovirus system. Bielefeldt-Ohmann H; Beasley D W; Fitzpatrick D R; Aaskov J G. (Centre for Molecular Biotechnology, School of Life Science, Queensland University of Technology, Brisbane, Australia.. helle@biosci.uq.edu.au) . JOURNAL OF GENERAL VIROLOGY, (1997 Nov) 78 (Pt 11) 2723-33. Journal code: 0077340. ISSN: 0022-1317.** Pub. country: ENGLAND: United Kingdom. Language: English.

AB In a step towards a tetravalent dengue virus subunit vaccine which is economical to produce, highly immunogenic and stable, a hybrid dengue virus envelope (E) protein molecule has been constructed. It consists of 36 amino acids from the membrane protein, the N-terminal 288 amino acids of the dengue-2 virus E protein plus amino acids 289-424 of the dengue-3 virus E protein. It has been engineered for secretory expression by fusion to a mellitin secretory **signal sequence** and truncation of the hydrophobic transmembrane segment. Using the baculovirus expression system and serum-free conditions, more than 95% of recombinant dengue-2 virus-dengue-3 virus hybrid E protein (rD2D3E) was secreted into the cell culture supernatant in a stable form with multiple features indicative of preserved conformation. The hybrid molecule reacted with a panel of dengue virus- and flavivirus-specific MAbs which recognize linear or conformational epitopes on dengue virions. Human dengue virus-specific antisera also reacted with the protein. The hybrid rD2D3E protein was able to inhibit the in vitro binding of dengue-2 and dengue-3 viruses to human myelomonocytic cells, suggesting that the receptor-binding epitope(s) was preserved. Adjuvant-free immunization with the hybrid protein induced an antibody response to both dengue-2 and dengue-3 virus in outbred mice, comparable in strength to that of individual rD2E and rD3E proteins. Notably, these antibody responses were primarily of the IgG2a and IgG2b isotype. A strong dengue virus cross-reactive T cell response was also induced in the mice, suggesting that dengue virus hybrid E proteins could form the basis of an efficacious multivalent

dengue virus vaccine.

L16 ANSWER 6 OF 17 MEDLINE on STN
97037001 Document Number: 97037001. PubMed ID: 8882648. Dengue virus envelope glycoprotein can be secreted from insect cells as a fusion with the maltose-binding protein. Staropoli I; Clement J M; Frenkiel M P; Hofnung M; Deubel V. (Unite des Arbovirus et Virus des Fievres Hemorragiques, Institut Pasteur, Paris, France.) JOURNAL OF VIROLOGICAL METHODS, (1996 Feb) 56 (2) 179-89. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB The maltose-binding protein (MalE) contains a signal sequence which allows its translocation in the periplasm of prokaryotic microorganisms. In this study, MalE was produced in Spodoptera frugiperda (Sf9) lepidopterian cells using the baculovirus expression system. The secretion of MalE, following cleavage of its signal sequence, to the supernatant fluid of recombinant baculovirus-infected Sf9 cells and its affinity for maltodextrin polymers allowed recovery of significant amounts (> or = 10 micrograms per 10(6) cells) of highly purified protein. The gene encoding the envelope glycoprotein E of the dengue (DEN) type 2 virus deleted of its C-terminal 102 amino acids (D2E delta 102) was fused to the MalE gene. The resulting hybrid MalE-D2E delta 102 glycoprotein was processed through the Golgi network of Sf9 cells and was secreted. It was retained on a maltodextrin column and was eluted with maltose. Antigenic and immunogenic properties dependent on the three-dimensional structure in the native E protein were preserved in the recombinant MalE-D2E delta 102 protein. Thus MalE with its signal sequence may be used as a carrier protein for production in the baculovirus system and purification of proteins which require transportation through intracellular compartments for correct folding and processing.

L16 ANSWER 7 OF 17 MEDLINE on STN
94335092 Document Number: 94335092. PubMed ID: 8057458. **Processing of the intracellular form of the west Nile virus capsid protein by the viral NS2B-NS3 protease: an in vitro study. Yamshchikov V F; Compans R W. (Department of Microbiology and Immunology, Emory University School of Medicine, Emory University, Atlanta, Georgia 30322.) JOURNAL OF VIROLOGY, (1994 Sep) 68 (9) 5765-71. Journal code: 0113724. ISSN: 0022-538X.** Pub. country: United States. Language: English.

AB According to the existing model of flavivirus polyprotein processing, one of the cleavages in the amino-terminal part of the flavivirus polyprotein by host cell signalases results in formation of prM (precursor to one of the structural proteins, M) and the membrane-bound intracellular form of the viral capsid protein (Cint) retaining the **prM signal sequence** at its carboxy terminus. This **hydrophobic anchor** is subsequently removed by the viral protease, resulting in formation of the mature viral capsid protein found in virions (Cvir). We have prepared **in vitro expression cassettes** coding for both forms of the capsid protein, for the prM protein, for the C-prM precursor, and for the viral protease components of West Nile flavivirus and characterized their translation products. Using Cint and Cvir translation products as molecular markers, we have observed processing of the intracellular form of the West Nile capsid protein by the viral protease in vitro both upon cotranslation of the C-prM precursor and the viral protease-encoding cassette and by incubation of C-prM translation products with a detergent-solubilized extract of cells infected with a recombinant vaccinia virus expressing the active viral protease. The cleavage of Cint by the viral protease at the predicted dibasic site was verified by introduction of point mutations

into the cleavage site and an adjacent region. These studies provide the first direct demonstration of processing of the intracellular form of the flavivirus capsid protein by the viral protease.

L16 ANSWER 8 OF 17 MEDLINE on STN
94246724 Document Number: 94246724. PubMed ID: 8189517. NS2B-3 proteinase-mediated processing in the yellow fever virus structural region: in vitro and in vivo studies. Amberg S M; Nestorowicz A; McCourt D W; Rice C M. (Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110-1093.) JOURNAL OF VIROLOGY, (1994 Jun) 68 (6) 3794-802. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Several of the cleavages required to generate the mature nonstructural proteins from the flaviviral polyprotein are known to be mediated by a complex consisting of NS2B and a serine proteinase domain located in the N-terminal one-third of NS3. These cleavages typically occur after two basic residues followed by a short side chain residue. Cleavage at a similar dibasic site in the structural region is believed to produce the C terminus of the virion capsid protein. To study this cleavage, we developed a cell-free trans cleavage assay for yellow fever virus (YF)-specific proteolytic activity by using a substrate spanning the C protein dibasic site. Cleavage at the predicted site was observed when the substrate was incubated with detergent-solubilized lysates from YF-infected BHK cells. NS2B and the NS3 proteinase domain were the only YF-specific proteins required for this cleavage. Cell fractionation studies demonstrated that the YF-specific proteolytic activity was membrane associated and that activity could be detected only after detergent solubilization. Previous cell-free studies led to a hypothesis that processing in the C-prM region involves (i) translation of C followed by translocation and core glycosylation of prM by using an internal signal sequence, (ii) signalase cleavage to produce a membrane-anchored form of the C protein (anchC) and the N terminus of prM, and (iii) NS2B-3-mediated cleavage at the anchC dibasic site to produce the C terminus of the virion C protein. However, the results of in vivo transient-expression studies do not support this temporal cleavage order. Rather, expression of a YF polyprotein extending from C through the N-terminal one-third of NS3 revealed that C-prM processing, but not translocation, was dependent on an active NS2B-3 proteinase. This suggests that signalase-mediated cleavage in the lumen of the endoplasmic reticulum may be dependent on prior cleavage at the anchC dibasic site. Possible pathways for processing in the C-prM region are outlined and discussed.

L16 ANSWER 9 OF 17 MEDLINE on STN
93134804 Document Number: 93134804. PubMed ID: 8421901. Mutagenesis of conserved residues at the yellow fever virus 3/4A and 4B/5 dibasic cleavage sites: effects on cleavage efficiency and polyprotein processing. Lin C; Chambers T J; Rice C M. (Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110-1093.) VIROLOGY, (1993 Feb) 192 (2) 596-604. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Flavivirus proteins are produced by co- and post-translational proteolytic processing of a large polyprotein using both host- and virus-encoded enzymes. The flavivirus serine proteinase, which consists of NS2B and NS3, is responsible for cleavages of at least four dibasic sites in the nonstructural region. In this study, a number of substitutions for the conserved amino acids flanking the 3/4A and 4B/5 dibasic cleavage sites

[Arg(P2)-Arg(P1) decreases Gly(P1')] were examined for their effects on yellow fever virus (YF) polyprotein processing. The substrate for these studies was a truncated YF polyprotein, called sig2A-5(356), which consists of a signal sequence fused to NS2A and extending through the first 356 amino acids of NS5. At the P1' position (Gly) of the 4B/5 site, only Ser and Ala were allowed while six other substitutions abolished cleavage. Substitutions of the 4B/5 P1 Arg residue with Lys, Gln, Asn, or His were tolerated while replacement with Glu eliminated cleavage. The 4B/5 P2 position (Arg) was found to be tolerant of substitutions with polar or hydrophobic residues which allowed varying degrees of partial cleavage. Previous studies have shown that cleavage at the 3/4A site is incomplete in YF-infected cells and that the cleavage efficiency at this site is significantly less for the sig2A-5(356) polyprotein. Replacement of the 3/4A P1 Arg residue with noncharged polar or hydrophobic residues reduced the cleavage efficiency, whereas substitutions with Glu or Pro abolished cleavage. Studies with polyproteins containing one or both of the 3/4A and 4B/5 cleavage sites blocked indicate that there is not an obligatory processing order for cleavages generating the N termini of YF NS4A, NS4B, and NS5.

L16 ANSWER 10 OF 17 MEDLINE on STN
93079885 Document Number: 93079885. PubMed ID: 1448926. **Expression and secretion of Japanese encephalitis virus nonstructural protein NS1 by insect cells using a recombinant baculovirus. Flamand M; Deubel V; Girard M. (Laboratoire des Arbovirus, Institut Pasteur, Paris, France.) VIROLOGY, (1992 Dec) 191 (2) 826-36.** Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The nonstructural protein NS1 of **Japanese encephalitis virus (JEV)** was expressed at a high level under the control of the polyhedrin promoter in Spodoptera frugiperda (Sf9) insect cells using a recombinant baculovirus. Recombinant NS1 was designed to contain its natural **signal sequence** at its N-terminus and no C-terminal hydrophobic domain that could act as a membrane anchor. This recombinant protein exhibited similar size to native NS1 expressed in Aedes albopictus (C6/36) insect cells infected with wild-type JEV. The signal sequence of NS1 allowed translocation of the protein into the endoplasmic reticulum where it underwent glycosylation. A small fraction of synthesized NS1 was able, in the absence of any other viral protein, to associate as a homodimer, showing similar characteristics to the native dimer. Interestingly, this recombinant dimeric form seemed to be exported and released in the extracellular medium of infected cell culture. During its transport, one of the two N-linked oligosaccharides of the polymannose type was processed to an endoglycosidase H-resistant form, suggesting that the protein had passed through the Golgi compartment before reaching the cell surface. Moreover, Triton X-114 partitioning analysis showed that monomeric NS1 behaved essentially as a hydrophilic protein, whereas both intracellular and extracellular dimeric NS1 were either free of or associated to membraneous components.

L16 ANSWER 11 OF 17 MEDLINE on STN
92148939 Document Number: 92148939. PubMed ID: 1531368. Cleavage of the dengue virus polyprotein at the NS3/NS4A and NS4B/NS5 junctions is mediated by viral protease NS2B-NS3, whereas NS4A/NS4B may be processed by a cellular protease. Cahour A; Falgout B; Lai C J. (Molecular Viral Biology Section, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.) JOURNAL OF VIROLOGY, (1992 Mar) 66

(3) 1535-42. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The cleavage mechanism utilized for processing of the NS3-NS4A-NS4B-NS5 domain of the dengue virus polyprotein was studied by using the vaccinia virus expression system. Recombinant vaccinia viruses vNS2B-NS3-NS4A-NS4B-NS5, vNS3-NS4A-NS4B-NS5, vNS4A-NS4B-NS5, and vNS4B-NS5 were constructed. These recombinants were used to infect cells, and the labeled lysates were analyzed by immunoprecipitation. Recombinant vNS2B-NS3-NS4A-NS4B-NS5 expressed the authentic NS3 and NS5 proteins, but the other recombinants produced uncleaved polyproteins. These findings indicate that NS2B is required for processing of the downstream nonstructural proteins, including the NS3/NS4A and NS4B/NS5 junctions, both of which contain a dibasic amino acid sequence preceding the cleavage site. The flavivirus NS4A/NS4B cleavage site follows a long hydrophobic sequence. The polyprotein NS4A-NS4B-NS5 was cleaved at the NS4A/NS4B junction in the absence of other dengue virus functions. One interpretation for this finding is that NS4A/NS4B cleavage is mediated by a host protease, presumably a signal peptidase. Although vNS3-NS4A-NS4B-NS5 expressed only the polyprotein, earlier results demonstrated that cleavage at the NS4A/NS4B junction occurred when an analogous recombinant, vNS3-NS4A-84%NS4B, was expressed. Thus, it appears that uncleaved NS3 plus NS5 inhibit NS4A/NS4B cleavage presumably because the putative signal sequence is not accessible for recognition by the responsible protease. Finally, recombinants that expressed an uncleaved NS4B-NS5 polyprotein, such as vNS4A-NS4B-NS5 or vNS4B-NS5, produced NS5 when complemented with vNS2B-30%NS3 or with vNS2B plus v30%NS3. These results indicate that cleavage at the NS4B/NS5 junction can be mediated by NS2B and NS3 in trans.

L16 ANSWER 12 OF 17 MEDLINE on STN
92109578 Document Number: 92109578. PubMed ID: 1729986. Processing of dengue virus type 2 structural proteins containing deletions in hydrophobic domains. Gruenberg A; Wright P J. (Department of Microbiology, Monash University, Clayton, Victoria, Australia.) ARCHIVES OF VIROLOGY, (1992) 122 (1-2) 77-94. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB The 5' end of the genome of the dengue virus type 2 encoding the structural proteins was expressed using recombinant vaccinia virus. Three additional recombinants derived by deletion of selected dengue sequences within the parental construct were also expressed. They were designed to assess the role of hydrophobic domains in the processing of the viral polyprotein in intact cells. The first construct contained a deletion of nucleotides encoding most of the C protein; nucleotides encoding the hydrophobic domain at the carboxy terminus were retained. The second and third constructs contained smaller deletions of 72 bp and 129 bp encoding hydrophobic domains at the carboxy termini of C and prM respectively. Indirect immunofluorescence and radioimmunoprecipitation were used to detect prM and E in cells infected with recombinant viruses. The results showed that deletion of 90% of C had no apparent effect on the processing of prM and E, and that the signal sequence for E at the carboxy terminus of prM was active in the absence of the upstream signal sequence for prM at the carboxy terminus of C. Deletion of the hydrophobic sequences preceding the amino terminus of E prevented cleavage at the prM-E junction. These results obtained using infected cells were consistent with the published findings for the translation of flavivirus RNA in vitro, and indicated the importance of membrane association in the cleavage of structural proteins from the flavivirus polyprotein. In addition, cells infected with the recombinant virus containing the large deletion in the C coding region

released the E glycoprotein into the culture medium.

L16 ANSWER 13 OF 17 MEDLINE on STN
92015500 Document Number: 92015500. PubMed ID: 1833562. **Processing of the yellow fever virus nonstructural polyprotein: a catalytically active NS3 proteinase domain and NS2B are required for cleavages at dibasic sites. Chambers T J; Grakoui A; Rice C M. (Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110-1093.) JOURNAL OF VIROLOGY, (1991 Nov) 65 (11) 6042-50. Journal code: 0113724. ISSN: 0022-538X.** Pub. country: United States. Language: English.

AB The vaccinia virus-T7 transient expression system was used to further examine the role of the NS3 proteinase in processing of the yellow fever (YF) virus nonstructural polyprotein in BHK cells. YF virus-specific polyproteins and cleavage products were identified by immunoprecipitation with region-specific antisera, by size, and by comparison with authentic YF virus polypeptides. A YF virus polyprotein initiating with a **signal sequence** derived from the E protein fused to the N terminus of NS2A and extending through the N-terminal 356 amino acids of NS5 exhibited processing at the 2A-2B, 2B-3, 3-4A, 4A-4B, and 4B-5 cleavage sites. Similar results were obtained with polyproteins whose N termini began within NS2A (position 110) or with NS2B. When the NS3 proteinase domain was inactivated by replacing the proposed catalytic Ser-138 with Ala, processing at all sites was abolished. The results suggest that an active NS3 proteinase domain is necessary for cleavage at the diabasic nonstructural cleavage sites and that cleavage at the proposed 4A-4B signalase site requires prior cleavage at the 4B-5 site. Cleavages were not observed with a polyprotein whose N terminus began with NS3, but cleavage at the 4B-5 site could be restored by supplying the the NS2B protein in trans. Several experimental results suggested that trans cleavage at the 4B-5 site requires association of NS2B and the NS3 proteinase domain. Coexpression of different proteinases and catalytically inactive polyprotein substrates revealed that trans cleavage at the 2B-3 and 4B-5 sites was relatively efficient when compared with trans cleavage at the 2A-2B and 3-4A sites.

L16 ANSWER 14 OF 17 MEDLINE on STN
91202121 Document Number: 91202121. PubMed ID: 1826736. In vitro synthesis of West Nile virus proteins indicates that the amino-terminal segment of the NS3 protein contains the active centre of the protease which cleaves the viral polyprotein after multiple basic amino acids. Wengler G; Czaya G; Farber P M; Hegemann J H. (Institut fur Virologie, Justus-Liebig-Universitat Giessen, Germany.) JOURNAL OF GENERAL VIROLOGY, (1991 Apr) 72 (Pt 4) 851-8. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A virus-encoded protease that cleaves after multiple basic amino acid residues has been implicated in the processing of the flavivirus polyprotein. Recently, a computer search of amino acid residues which might form the active site of a protease led to the suggestion that the amino-terminal segment of the NS3 protein represents a serine protease. To examine this possibility we constructed an mRNA which encodes a polyprotein with an amino-terminal signal sequence derived from the influenza virus haemagglutinin, followed by a segment of the West Nile flavivirus polyprotein which includes the non-structural (NS) proteins NS2A, NS2B and the amino-terminal part of the NS3 protein. This polyprotein contains two sequences, located at the termini of the NS2B protein, which are cleaved by the viral protease that cleaves after

multiple basic residues in the authentic polyprotein. The proteins that are generated by this mRNA during in vitro translation in the presence of rough endoplasmic reticulum membranes indicate that these two proteolytic cleavages occur in vitro. In vitro translation of polyproteins shortened at the carboxy terminus shows that a polyprotein which does not contain the complete set of proposed catalytic residues present in the NS3 protein segment accumulates as a membrane-associated molecule without proteolytic processing. Similarly, substitution of residue histidine 51 of the NS3 polyprotein segment, which is predicted to be part of the protease catalytic centre, with an alanine residue, blocks the processing of the polyprotein in vitro.

L16 ANSWER 15 OF 17 MEDLINE on STN
89311624 Document Number: 89311624. PubMed ID: 2501515. **In vitro processing of dengue virus structural proteins: cleavage of the pre-membrane protein. Markoff L. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.) JOURNAL OF VIROLOGY, (1989 Aug) 63 (8) 3345-52. Journal code: 0113724. ISSN: 0022-538X.** Pub. country: United States. Language: English.

AB Processing of dengue virus structural proteins was assessed in vitro. RNA transcripts for cell-free translation were prepared from cloned DNA (dengue virus type 4, strain 814669 genome) encoding capsid, pre-membrane (prM), and the first 23 amino acids of envelope (E). Processing of a 33-kilodalton precursor polypeptide encoded by wild-type RNA transcripts occurred only in the presence of added microsomal membranes. Under these conditions, cleavage at the capsid-prM and prM-E sites and glycosylation of prM occurred in association with translocation. Amino acid sequence analysis confirmed that translation initiated at the predicted N terminus of the capsid and that capsid-prM cleavage occurred at the predicted site for the action of signal peptidase following a **candidate signal sequence (hydrophobic residues 100 to 113) in the dengue virus precursor.** Mutations were introduced into the dengue virus DNA template by site-directed mutagenesis, altering nucleotide sequences encoding the capsid and the candidate signal for prM. The phenotypes of the mutants were deduced by analysis of the products of cell-free translation of the respective RNA transcripts. The resulting observations confirmed that cleavage at the capsid-prM and prM-E sites is effected entirely by signal peptidase and that the candidate signal is required for translocation.

L16 ANSWER 16 OF 17 MEDLINE on STN
89199735 Document Number: 89199735. PubMed ID: 2522997. **Proper processing of dengue virus nonstructural glycoprotein NS1 requires the N-terminal hydrophobic signal sequence and the downstream nonstructural protein NS2a.** Falgout B; Chanock R; Lai C J. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.) JOURNAL OF VIROLOGY, (1989 May) 63 (5) 1852-60. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Expression of dengue virus gene products involves specific proteolytic cleavages of a precursor polyprotein. To study the flanking sequences required for expression of the dengue virus nonstructural glycoprotein NS1, we constructed a series of recombinant vaccinia viruses that contain the coding sequence for NS1 in combination with various lengths of upstream and downstream sequences. The NS1 products expressed by these viruses in infected CV-1 cells were

immune precipitated and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The data show that the 24-residue hydrophobic sequence preceding NS1 was necessary and sufficient for the production of glycosylated NS1 and that this sequence was cleaved from NS1 in the absence of most dengue virus proteins. This finding is consistent with previous proposals that this **hydrophobic sequence serves as an N-terminal signal sequence** that is cleaved by signal peptidase. The cleavage between the C terminus of NS1 and the downstream protein NS2a occurred when the complete NS2a was present. Recombinant viruses containing NS1 plus 15 or 49% of NS2a produced proteins larger than authentic NS1, indicating that the cleavage between NS1 and NS2a had not occurred. Failure of cleavage was not corrected by coinfection with a recombinant virus capable of cleavage. These results suggest that NS2a may be a cis-acting protease that cleaves itself from NS1, or NS2a may provide sequences for recognition by a specific cellular protease that cleaves at the NS1-NS2a junction.

L16 ANSWER 17 OF 17 MEDLINE on STN
87293881 Document Number: 87293881. PubMed ID: 3039728. The nucleotide sequence of dengue type 4 virus: analysis of genes coding for nonstructural proteins. Mackow E; Makino Y; Zhao B T; Zhang Y M; Markoff L; Buckler-White A; Guiler M; Chanock R; Lai C J. VIROLOGY, (1987 Aug) 159 (2) 217-28. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB We recently cloned a full-length DNA copy of the dengue type 4 virus genome. Analysis of the 5' terminal nucleotide sequence suggested that the three-virion structural proteins are synthesized by proteolytic cleavage of a polyprotein precursor which is encoded in one open reading frame. We now present the remaining sequence of the dengue type 4 virus genome which codes for the nonstructural proteins. The entire genome, which is 10,644 nucleotides in length, contains one long open reading frame which codes for a single large polyprotein 3386 amino acids in length. Alignment of the dengue nonstructural protein sequence with that of other flaviviruses, including yellow fever and West Nile viruses, revealed that significant homology exists throughout the entire nonstructural region of the dengue genome and this allowed tentative assignment of individual nonstructural proteins in the following order: NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5-COOH. Processing of the nonstructural proteins appears to involve two types of proteolytic cleavage: the first occurs after a **long hydrophobic signal sequence** and the second occurs at a junction between two basic amino acids and a small polar amino acid. A notable exception is the cleavage at the N-terminus of the dengue NS3 which may take place at the junction between Gln-Arg and Ser. Comparative analysis suggests that dengue NS3 and NS5 may be involved in enzymatic activities related to viral replication and/or transcription. Putative nonstructural proteins NS2a, NS2b, NS4a, and NS4b are extremely hydrophobic, suggesting that these proteins are most likely associated with cellular membranes.

FILE LAST UPDATED: 19 Feb 2004 (20040219/ED)
HIGHEST GRANTED PATENT NUMBER: US6694518
HIGHEST APPLICATION PUBLICATION NUMBER: US2004034897
CA INDEXING IS CURRENT THROUGH 19 Feb 2004 (20040219/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 19 Feb 2004 (20040219/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Dec 2003
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2003

>>> USPAT2 is now available. USPATFULL contains full text of the <<<
>>> original, i.e., the earliest published granted patents or <<<
>>> applications. USPAT2 contains full text of the latest US <<<
>>> publications, starting in 2001, for the inventions covered in <<<
>>> USPATFULL. A USPATFULL record contains not only the original <<<
>>> published document but also a list of any subsequent <<<
>>> publications. The publication number, patent kind code, and <<<
>>> publication date for all the US publications for an invention <<<
>>> are displayed in the PI (Patent Information) field of USPATFULL <<<
>>> records and may be searched in standard search fields, e.g., /PN, <<<
>>> /PK, etc. <<<

>>> USPATFULL and USPAT2 can be accessed and searched together <<<
>>> through the new cluster USPATALL. Type FILE USPATALL to <<<
>>> enter this cluster. <<<
>>> <<<
>>> Use USPATALL when searching terms such as patent assignees, <<<
>>> classifications, or claims, that may potentially change from <<<
>>> the earliest to the latest publication. <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

```
=> s yellow fever virus
        209552 YELLOW
         15811 FEVER
         70100 VIRUS
L1         661 YELLOW FEVER VIRUS
                (YELLOW(W)FEVER(W)VIRUS)

=> s l1 and (yellow fever virus/clm)
         12294 YELLOW/CLM
          1051 FEVER/CLM
         11930 VIRUS/CLM
            59 YELLOW FEVER VIRUS/CLM
                ((YELLOW(W)FEVER(W)VIRUS)/CLM)
L2          59 L1 AND (YELLOW FEVER VIRUS/CLM)

=> s l2 and (prM and E)
          1268 PRM
       2292897 E
L3          17 L2 AND (PRM AND E)

=> s l3 and (prM/clm)
            44 PRM/CLM
L4           4 L3 AND (PRM/CLM)

=> d l4,cbib,1-4

L4   ANSWER 1 OF 4  USPATFULL on STN
2004:12950 Novel flavivirus antigens.
    Apt, Doris, Sunnyvale, CA, UNITED STATES
    Punnonen, Juha, Belmont, CA, UNITED STATES
    Brinkman, Alice M., Lake Bluff, IL, UNITED STATES
    Maxygen, Inc. Patent Department, Redwood City, CA, UNITED STATES (U.S.
    corporation)
    US 2004009469 A1 20040115
    APPLICATION: US 2003-375932  A1  20030226 (10)
```